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Immunocytochemical Identification of Human Chorionic Gonadotropin- and Alpha-Fetoprotein-Producing Cells of Hepatoblastoma Associated with Precocious Puberty

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Summary. A one-year-five-month-old boy with hepatoblastoma producing both human chorionic gonadotropin (hCG) and alpha-fetoprotein (AFP) is presented. Histologically, the primary tumor was mainly composed of well differentiated hepatoblastoma cells, with minor areas of poorly differentiated cells. Immunoperoxidase staining of the tumor for hCG and AFP showed that a few well differentiated, fetal type cells and multinucleated giant cells were positive for hCG, and AFP was never stained in the same cells. In areas where cells were poorly differentiated, positive reactions for either hCG or AFP were not observed. Electron microscopic studies revealed focal aggregates of cytoplasmic cored vesicles in some tumor cells, similar to secretory granules.

Key word: Hepatoblastoma – Gonadotropin – Alpha-fetoprotein – Immunoperoxidase staining

Ectopic production of human chorionic gonadotropin (hCG) by hepatoblastoma cells with associated precocious puberty is very rare. Since 1931, 17 cases of virilizing hepatoblastoma have been reported (Behrendt 1931; MacNab et al. 1952; Jolly 1955; Reeves et al. 1959; McArthur et al. 1973; Rossi et al. 1960; Hung et al. 1963; Behrle et al. 1963; Lipsett et al. 1964; Thamdrup 1965; Kosenow et al. 1967; Root et al. 1968; Schiødt 1970; Braunstein et al. 1972; Kumar et al. 1978), and increased levels of serum or urinary gonadotropin have been identified in only 10 such cases (Reeves et al. 1959; Rossi et al. 1960; Hung et al. 1963; Behrle et al. 1963; Thamdrup 1965; Kosenow et al. 1967; Root et al. 1968; Braunstein et al. 1972; McArthur et al. 1973; Kumar et al. 1978). Recent reports have further showed that some cases of hCG-producing hepatoblastoma are also accompanied by an increased production of alpha-fetoprotein (AFP) (Braunstein et al. 1972; Kumar et al. 1978). Braunstein et al. have reported a case of hepatoblastoma producing both AFP and hCG, which showed that after

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recurrence of the tumor, chemotherapy and radiotherapy resulted in a decrease in hCG levels but had no effect on AFP levels (Braunstein et al. 1972). Production of hCG or AFP by hepatoblastoma cells is clinically associated with a poor prognosis. However, the specific cells responsible for the production of hCG or AFP have not as yet been identified.

In this communication, we report a case of virilizing hepatoblastoma associated with high levels of both hCG and AFP. We have found by using the immunoperoxidase staining method, that while both hCG and AFP were produced by well differentiated tumor cells, no single cell was found to contain both substances.

Case Report

A one-year-five-month-old boy was admitted to the Kyushu University Hospital because of abdominal mass and precocious puberty. He developed normally until 5 months prior to admission. when it was noted that the child's penis was enlarging and pubic hair began to develop. Pigmentation of the scrotum and facial acne were also noted. His bone age at admission was $2^1/_2$ years. A liver scintigram and angiography showed a large lesion intrinsic to the right hepatic lobe. Suspecting hCG-producing hepatoblastoma, an extended right hepatic lobectomy was performed on the third day after admission. Preoperative laboratory findings were as follows: serum lactate dehydrogenase, 618 U/l; total serum cholesterol, 415 mg/dl; urinary 17-ketosteroid, 0.19 mg/24 h; serum AFP, 2,503,900 ng/ml; serum hCG, 606 mIU/ml; urinary hCG, 448.78 IU/24 h; serum and urinary testosterone, 3.97 ng/ml and 14.5 μg/24 h, respectively; urinary estriol, 47.5 μg/24 h; adrenocorticotropic hormone, 190 pg/ml; cortisol, 259 ng/ml; carcinoembryonic antigen (CEA), 1.1 ng/ml. The size of the resected tumor was $21 \times 10.5 \times 14$ cm, and the weight 1,610 g. The contents of hCG and AFP within the tumor were 72.6 mIU/g wet weight and 210.37 ng/g wet weight, respectively. Histologically, the tumor was mainly a well differentiated hepatoblatoma. Testicular biopsy was performed on the 12th postoperative day, and histological examinations showed neither obvious Leidig cell hyperplasia nor spermatogenesis. Chemotherapy, using vincristine and cyclophosphamide was started immediately after the operation and in addition, OK432 (Sankyo Pharmaceut. Co., Tokyo, Japan) was given as an immunopotentiator. Radiation therapy (total 1,220 Rad) was delivered to the mediastinal region in order to prevent metastasis (Fig. 4). Three months after surgery, the patient was alive without any obvious sign of recurrence. Later, local recurrence and lung and lymph node metastases developed.

Materials and Methods

Light and Electron Microscopes

For light microscopy, tissue was fixed in formalin solution, embedded in paraffin and cut and stained with haematoxylin and eosin. For electron microscopy, the fresh tissue for the primary tumor was fixed in 3% glutaraldehyde solution (buffered at pH 7.4) and postfixed in 1% phosphate-buffered osmium tetroxide. Following dehydration, the tissue blocks were embedded in Epon 812 and were cut on an LKB ultrotome III. Ultrathin sections were stained with uranyl acetate and lead citrate, and examined with a JEM 100C electron microscope.

Immunoperoxidase Stainings of hCG and AFP

Nine different portions of the primary tumor were fixed with 10% buffered formalin and embedded in paraffin. Sections 5 to 6 μm thick were deparaffinized, rinsed in cold phosphate buffered saline (PBS), incubated with rabbit antisera (anti-hCG beta diluted 1:20–40, anti-AFP diluted 1:20, both received from Dr. H. Okumura, Research Laboratories, Teikoku Mfg. Co. Ltd., Kawasaki) for 60 min at 37° C, and then with horseradish peroxidase (HRP)-labelled

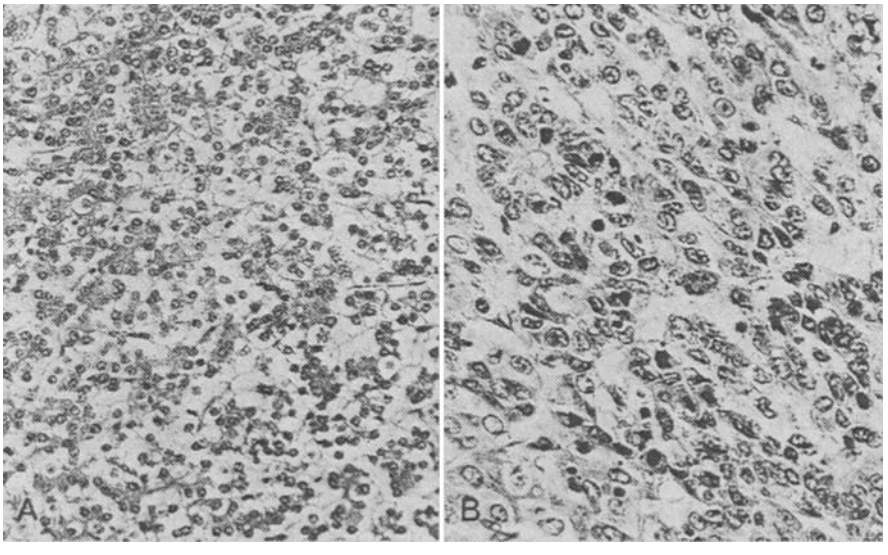

Fig. 1. A *(left)* light micrograph of the primary tumor showing well differentiated hepatoblastoma. The tumor cells show a low nuclear-to-cytoplasmic ratio and broad cytoplasm (H&E, ×180). B *(right)* Small areas of the primary tumor showing poorly differentiated hepatoblastoma. The fusiform and polygonal cells with little cytoplasm are arranged in sheets or rosettes (H&E, ×360)

antirabbit gammaglobulin goat gammaglobulin for 60 min at room temperature. The immunoperoxidase-stained sections were further reacted with 3,3'-diaminobenzidine with 0.005% hydrogen peroxide (Karnovsky) for 15 min at room temperature and examined under a conventional microscope. As a control, another set of sections was incubated with normal rabbit serum in place of the primary antisera.

Results

Histology and Immunoperoxidase Stainings for hCG and AFP

The majority of the cells of the primary tumor had characteristics of a well differentiated type (fetal type) of hepatoblastoma (Fig. 1A), with a minority of poorly differentiated cells (embryonal type) (Fig. 1B). The fetal type tumor cells were arranged in sinusoids, sheets and cords. Such polygonal tumor cells were large with abundant eosinophilic and granular cytoplasm. The hyperchromatic nuclei were relatively regular in both size and shape and the nuclear-to-cytoplasmic ratio was low. Mitotic figures were occasionally seen. Embryonal type tumor cells were smaller and fusiform, with hyperchromatic nuclei and scant cytoplasm, and were often arranged in ribbons or rosettes. Vascular lakes were scattered throughout the tumor. Foci of squamous epithelium, both with and without keratinization, were recognized.

The tumor cells stained by anti-AFP antibody labelled with peroxidase were well differentiated (Fig. 2A). The hCG-positive cells were also well

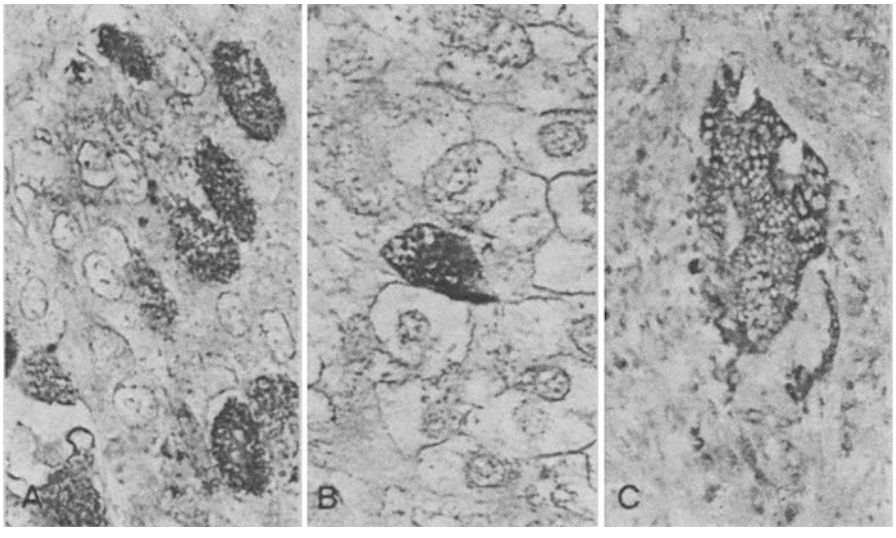

Fig. 2A–C. Immunoperoxidase staining of the primary tumor. **A** (*left*) Well differentiated tumor cells positive for AFP (×800). **B** (*center*) Well differentiated tumor cell positive for hCG (×800). **C** (*right*) Multinucleated giant cell positive for hCG (×390)

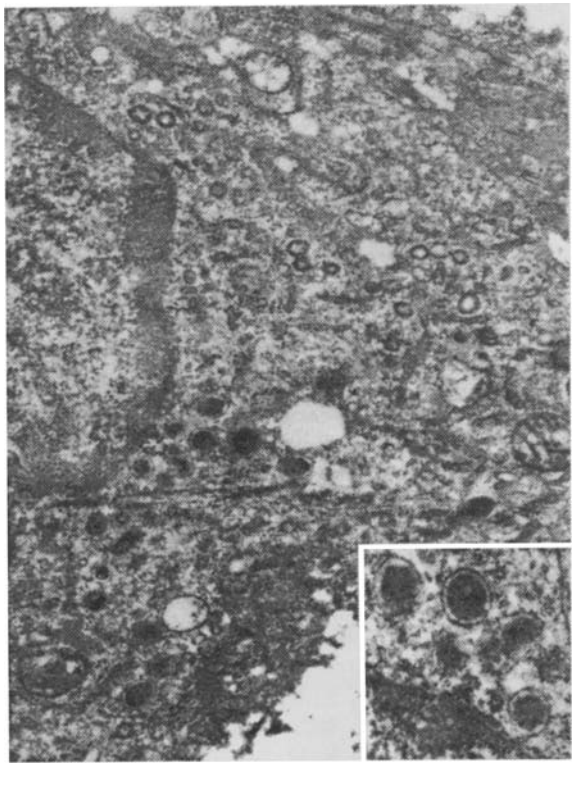

Fig. 3. Electron micrograph of a well differentiated tumor cell containing aggregates of intracytoplasmic cored vesicles (× 25,000). *Inset*: High magnification of cored vesicles (× 50,000)

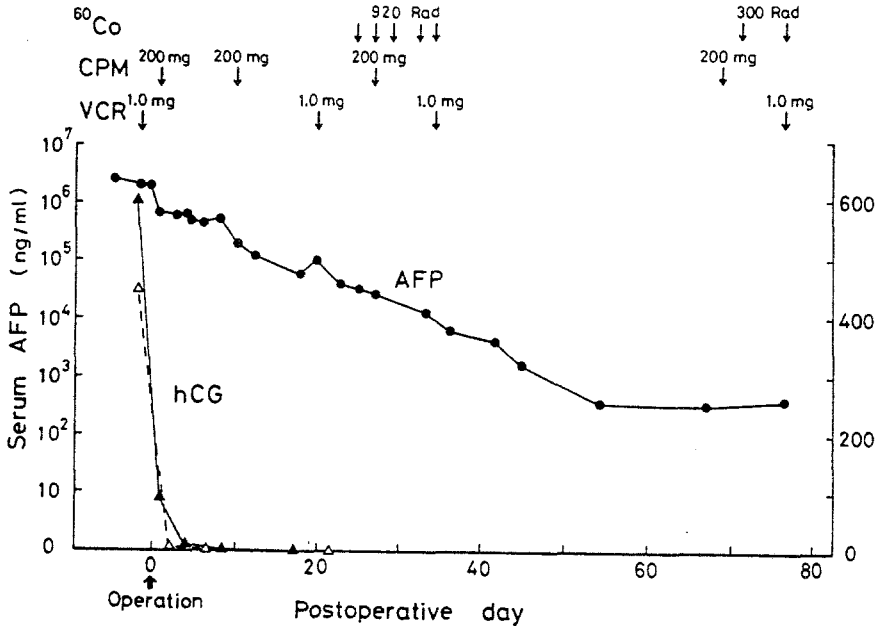

Fig. 4. Changes in AFP and hCG levels in serum and urine during hospital course. △—△ hCG in urine (IU/24 h); ▲—▲ hCG in serum (mIU/ml)

differentiated tumor cells, but the number was obviously smaller than that of AFP-positive cells (Fig. 2B). No cell was stained with both anti-AFP and anti-hCG antibodies labelled with peroxidase. The staining pattern of both AFP and hCG was granular and diffuse within the cells. Some multinucleated giant cells in the fibrous connective tissue were also stained by the labelled anti-hCG antibody, but not by the anti-AFP antibody (Fig. 2C).

Electron Microscopic Findings

The tumor cells had closely apposed simple plasma membrane (Fig. 3). Only rarely were desmosome-like junctions and microvillous-like interdigitations of cell membranes present. The basal laminae of the tumor cells were observed to about on the stroma. The cells contained few organelles, consisting mainly of aggregates of glycogen, short profiles of rough endoplasmic reticulum, small mitochondria and a few Golgi zones. Free ribosomes, lysosomal bodies, and microfilaments were diffusely dispersed throughout the cytoplasm. A striking finding was focal aggregates of intracytoplasmic cored vesicles (secretory type granules) present in some tumor cells (Fig. 3).

Postoperative Changes in hCG and AFP Levels

The serum level of hCG abruptly decreased from 606 mIU/ml to 8.4 mIU/ml within 5 days after resection of the hepatic tumor (50% decrease by 1.2 days), and decreased further to less than 1.0 mIU/ml 3 months after

the operation. Urinary hCG excretion also decreased rapidly from 448.78 IU/24 h to 5.73 IU/24 h by the fifth postoperative day. In contrast, the decrease in serum AFP after surgery was slow. A 50% decline was not observed until the fifth postoperative day, and significantly elevated levels (3,782 ng/ml) were still evident 3 months after removal of the primary tumor (Fig. 4).

Discussion

In the present study, the hepatoblastoma cells responsible for the production of hCG and AFP were directly identified by using immunoperoxidase staining methods. These studies have revealed that both hCG- and AFP-positive cells are well differentiated tumor cells. Furthermore, no single cell was stained by both anti-hCG and anti-AFP antibodies labelled by peroxidases, thus, hCG and AFP were probably secreted by different cells within the tumor. Another significant finding was that some multinucleated giant cells were also positive for hCG. We also observed electron microscopically secretory granule-like cored vesicles in some tumor cells which are very similar to those Kumar et al. recently reported (Kumar et al. 1978). However, further study is necessary to see if these vesicles represent secretory granules of hCG or AFP, or neither substance.

To our knowledge, 17 cases of hepatoblastoma associated with precocious puberty have been reported during the past half century (Behrendt 1931; MacNab et al. 1952; Jolly 1955; Reeves et al. 1959; Rossi et al. 1960; Hung et al. 1963; Behrle et al. 1963; Lipsett et al. 1964; Thamdrup 1965; Kosenow et al. 1967; Root et al. 1968; Schiødt 1970; Braunstein et al. 1972; McArthur et al. 1973; Kumar et al. 1978). In 1959, Reeves et al. showed that gonadotropin activities in one such patient's urine and tumor extract were markedly enhanced (Reeves et al. 1959). Since then, increased levels of hCG in serum and/or urine have been identified in 10 patients with virilizing hepatoblastoma (Reeves et al. 1959; Rossi et al. 1960; Hung et al. 1963; Behrle et al. 1963; Thamdrup 1965; Kosenow et al. 1967; Root et al. 1968; Braunstein et al. 1972; McArthur et al. 1973; Kumar et al. 1978).

Recently, Braunstein et al. reported an hCG-producing hepatoblastoma which concomitantly secreted AFP (Braunstein et al. 1972). They were able to detect the production of these proteins by tumor cells in an in vitro culture system, but they could not determine if both hCG and AFP were produced by the same cell. In addition, their patient's postoperative course showed the discordant behavior of the plasma hCG and AFP levels during chemotherapy and radiation therapy. A similar dissociation between hCG level and clinical course was also observed by Hung et al. (1963). Regarding this question, our immunohistochemical staining results suggest that such discordance might be due to the fact that different tumor cells produce each substance, and that AFP-producing cells may be able to establish metastatic foci after apparent remission. It is, of course, probable that hCG-producing cells have different sensitivity from AFP-producing cells to che-

motherapy or radiation therapy. Further consideration of this problem should be undertaken in the hope of producing more effective clinical approaches.

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